The Free Internet Journal for Organic Chemistry

Paper

Archive for Organic Chemistry

Arkivoc **2017**, part iv, 88-94

Kinetics of photochemical isomerization of TFA-Gly-^{Z}ΔPhe into TFA-Gly-^{E}ΔPhe

Maciej Makowski,[a] Michał Jewgiński,[b] Józef Hurek,[a] Anna Poliwoda,[a] and Paweł Kafarski[a,b]

[a] Faculty of Chemistry, University of Opole, Oleska 48, 45-052 Opole, Poland

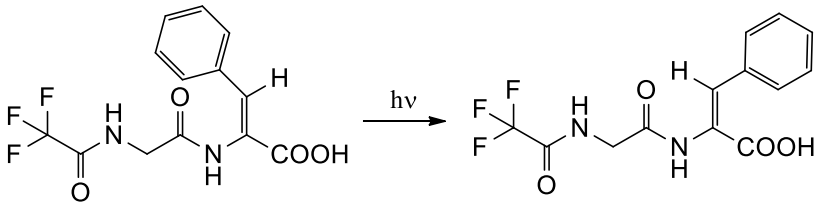
[b] Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

Email: Maciej.Makowski@uni.opole.pl; michal.jewgiński@pwr.edu.pl

Received 11-14-2016 **Accepted** 02-18-2017 **Published on line** 05-07-2017

Abstract

The kinetics of photoisomerization of trifluoroacetyl-(*Z*)-dehydrophenylalanylglycine into trifluoroacetyl-(*E*)-dehydrophenylalanylglycine was studied in the hope that light-induced reaction could be useful as a means of preparation of the *E*-dehydropeptides. The obtained results indicate that if this reaction carried out under irradiation with light of wavelength 360 nm it is practically irreversible and gave nearly quantitatively pure *E*-isomer Significantly, expected cyclic side-products were not observed in the reaction mixture, thus proving the preparative potential of the elaborated procedure.



TFA-Gly-^{Z}ΔPhe TFA-Gly-^{E}ΔPhe

Keywords: Dehydropeptides, photoisomerization, *E-Z* isomers, reaction kinetics, NMR

Introduction

Dehydroamino acids contribute to catalytic action in tyrosine aminomutase[1] and green fluorescent proteins.[2] They also occur in a variety of peptide antibiotics of microbial origin (including important lantibiotics such as: nisin, subtilin, epidermin and gallidermin),[3] neurotoxins (roquefortin, oxaline and phomopsin),[4] hepatotoxins (microcystins and nodularins),[5] and phytotoxic (tentoxin and AM toxins)[6,7] and antitumor agents (phenylahistin, telomestatin).[8,9]

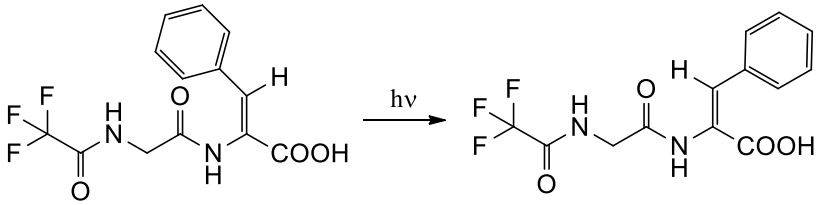
Dehydropeptides might be also considered as a class of promising foldamers[10] because of their ability to take specific three-dimensional structure forms resulting from the presence of a double bond between the Cα and Cβ atoms, which causes the coupling of this double bond with flanking amide fragments and results in rigid structure of the peptide chain.[11-14]

Numerous studies dealing with dehydropeptide structure and conformation have used ^{Z}ΔPhe containig peptides owing to their easy chemical synthesis and substantial stability upon storage,[15-18] whereas peptides containing ^{E}ΔPhe are scarcely described in the literature. This is because *E*-dehydroamino acids and their peptides are unstable and slowly undergo thermal isomerization upon conditions of peptide synthesis.[19,20]

In this paper we present a study of the kinetics of isomerization of a simple dipeptide derivative containing (*Z*)-dehydrophenylalanine (Gly-^{Z}ΔPhe) into its *E*-isomer.

Results and Discussion

TFA-Gly-^{Z}ΔPhe was converted into TFA-Gly-^{E}ΔPhe (Scheme) by constant irradiation of its benzene/acetone solution with UV light at 254 nm for 18 days at room temperature.



Scheme 1. Photochemical conversion of TFA-Gly-^{Z}ΔPhe into TFA-Gly-^{E}ΔPhe.

After fixed periods of time the percent of conversion was determined by means of ^{1}H NMR spectrometry using the spectra recorded in deuterated DMSO. Three regions of the spectra have been chosen for analysis: (i) intensity of signal derived from ^{E}ΔPhe amide proton (denoted as A in Figure 1); (ii) intensity of peaks representing amidic protons of ^{Z}ΔPhe and of two glycines present in both isomers (denoted as B in Figure 1); (iii) intensity of CH_2 region derived from both peptides (denoted as C in Figure 1A). Basing on these measurements relative quantities of both isomers were determined.

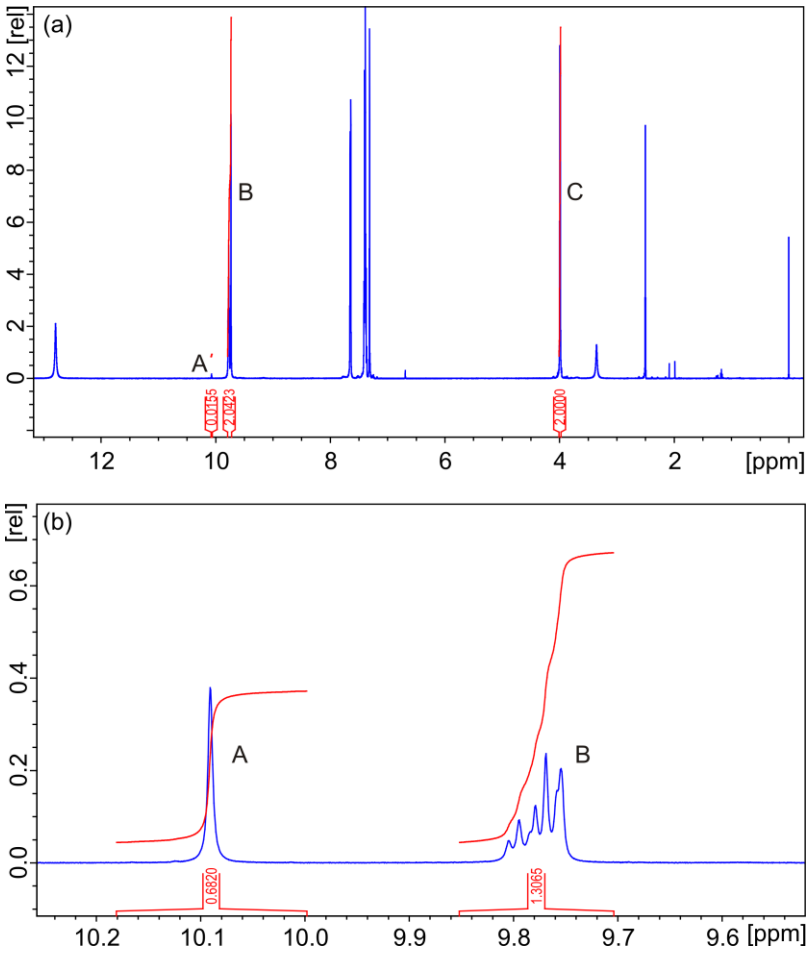


Figure 1. Representative 1H NMR spectra used to follow the course of isomerization: (a) full spectrum of the reaction mixture at first day of the process; (b) amidic region of spectrum taken after four days of process.

Basing on these determinations curves of progression of the reaction were found (Figure 2) showing quite symmetric increase in molar ratio of isomer *E* in the reaction mixture with simultaneous decrease of the share of isomer *Z*. These progression curves were analyzed by applying standard kinetic equations,[21] which unequivocally indicated that the studied reaction is of the first order, with k_1 value of (12.43 ± 0.27)×10^{-3} h^{-1} and k_{-1} of (1.71 ± 0.077) ×10^{-3} h^{-1} and R^2 = 0.996. Equilibrium of the reaction was reached after fifteen - twenty days of the process. The calculated reaction constant K was 7.26 ± 0.49 indicating seven-fold prevalence of the substrate in final reaction mixture.

To substantiate this finding TFA-Gly-^{Z}ΔPhe was also converted into into TFA-Gly-^{E}ΔPhe by constant irradiation of its benzene/acetone solution with light of different wavelength, namely 360 nm, for 80 hours at room temperature. In this case only the decrease in concentration of TFA-Gly-^{Z}ΔPhe was measured by means of HPLC because pure TFA-Gly-^{E}ΔPhe crystallizes from the mixture. Similar as above, analysis of the experimental results (Figure 3) indicated that in this case the reaction is practically irreversible with k =0.0458 ± 0.001943 h^{-1} and R^2 = 0.96. The irreversible character of this reaction clearly and easy isolation of pure TFA-Gly-^{E}ΔPhe in nearly quantitative yield shows that photochemical isomerization of readily available *Z*-dehydropeptides into their *E*-isomers has a preparative value.

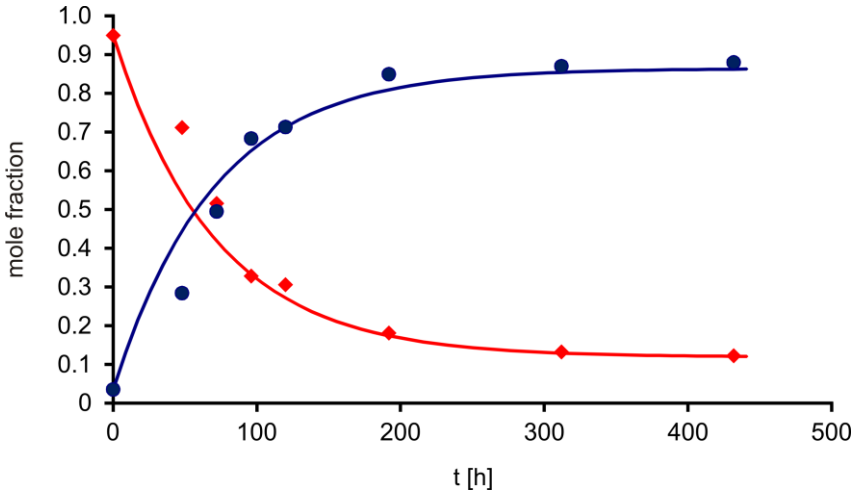


Figure 2. Experimentally (points) and theoretically (lines) determined courses of reaction: increase in concentration of TFA-Gly-^{E}ΔPhe (blue line) versus decrease in concentration of TFA-Gly-^{Z}ΔPhe (red line).

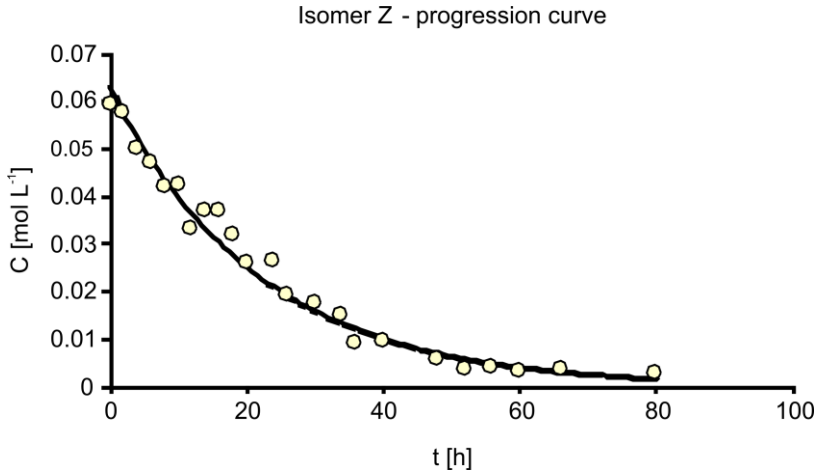


Figure 3. Experimentally (points) and theoretically (line) determined progression curve for conversion of TFA*Δ^{Z}Phe-Gly.

Preparative conversion of substituted ^{Z}ΔPhe into ^{E}ΔPhe,[22] as well as such isomerization of this dehydroamino acid in several peptides has been described previously.[23,24] However, these papers, dedicated to the photochemical reactions of slightly different aromatic dehydroamino acids, state that this process is not so simple and is accompanied by variable photochemical cyclizations.[25,26] Our study, however, does not indicate the presence of any other products than Gly-^{E}ΔPhe in the case of this photochemical reaction, and no cyclization products were found even after long irradiation of reaction mixture, irrespective of which analytical method was used.

Conclusions

Kinetic studies of the photo-conversion of TFA-Δ^{Z}Phe-Gly into TFA-Δ^{E}Phe-Gly showed that the equilibrium of process is strongly shifted towards the creation of *E*-isomer. Since the NMR studies did not show the presence of cyclization products, which have been reported in the literature, our studies indicate the preparative

usefulness of the light-induced isomerization of Z-dehydropeptide for obtaining peptides containing the Δ^{E}Phe residue. This was confirmed by the easy isolation of pure TFA-Δ^{E}Phe-Gly, which crystallizes from the reaction mixture.

Experimental Section

General. ^{1}H NMR spectra were recorded on a Brucker Avance II Ultrashield Plus 600 MHz (^{1}H: 600.58 MHz) using $SiMe_4$ as internal reference. The chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and Hertz respectively. The signal assignments were made using HSCQ spectra.

The samples were analyzed using Waters Model 2695 Alliance Separation Module HPLC System (Waters Corporation, Milford, Massachusetts, USA) equipped with 2487 Dual Absorbance Detector. Data acquisition and integration were performed using Clarity Chromatography software 6.1 (DataApex, Prague, Czech Republic). The studied compounds were separated on Ascentis® Express C18 ((100 mm × 4.6 mm, I.D. 2.7 µm) column protected by a Ascentis® Express C18, 2.7 µm guard cartridge (5 mm x 4.6 mm). The chromatographic separation was performed in isocratic mode, using the mixture of 0.1% CH_3COOH in water (solvent A) and acetonitrile (solvent B) with a ratio 75%A:25%B. The applied flow rate was 0.5 ml min^{-1}. The injection volume was 20 µl. The temperature of the HPLC oven and autosampler was set at 20 °C. The chromatograms were monitored at 280 nm.

Dehydropeptides. Dehydropeptides were available from previous studies.[12]

TFA-Δ^{Z}Phe-Gly: ^{1}H NMR(DMSO-d_6): δ=3.99 (5.95 2H, d); δ=7.31 (1H, s); δ=7.37÷7.42 (3H, m); δ=7.64÷7.66 (2H, m); δ=9.73 (1H, s); δ=9.77 (6.00 1H, t); δ=12.79 (1H, bs)

TFA-Δ^{E}Phe-Gly:: ^{1}H NMR(DMSO-d_6): δ=3.98 (6.01 2H, d); δ=6.70 (1H, s); δ=7.23÷7.25 (1H, m); δ=7.29÷7.33 (4H, m); δ=9.76 (6.06 1H, t); δ=10.08 (1H, s); δ13.02 (1H, bs)

Isomerization reaction monitored by ^{1}H NMR. Trifluoroacetyl-(*Z*)-glycyl-dehydrophenylalanine (0.050g) was dissolved in a mixture composed of 1.5 ml of benzene and 1.0 ml of acetone. Then 0.112 g of benzophenone (photosensitizer) was added and the reaction mixture irradiated with a 6W lamp at 254 nm. At certain periods of time the sample was evaporated under reduced pressure and its composition studied by ^{1}H NMR.

Isomerization reaction monitored by HPLC. In this case trifluoroacetyl-(*Z*)-glycyl-dehydrophenylalanine (3.162g; 10 mmole) was dissolved in mixture of 50 mL of acetone and 95 mL of benzene followed by addition of benzophenone (5.36g; 29.45 mmole) and the mixture irradiated with lamp at 360 nm. At fixed periods of time samples were studied by means of HPLC following the decrease in concentration of the substrate (after filtration of the formed isomer *E*). After reaction crystallized TFA-Gly-^{E}ΔPhe was separated by filtration (92.6% yield, m.p. 211-213 °C) while the substrate TFA-Gly-^{z}ΔPhe was isolated after evaporation of the solvents and crystallization from the mixture of ethyl acetate and hexane (6.2% yield, m.p. 194-196 °C).

Analysis of kinetic data

For the isomerization by irradiation of TFA-Δ^{Z}Phe-Gly with the wavelength of 254 nm first order reversible kinetic model was assumed as being the most suitable. In order to find kinetic parameters, concentrations of both substrate and product at equilibrium state had to be found. Using the function of linear regression, the best fit between theoretical and experimental curves have been obtained and thus kinetic parameters of the reaction were determined.

Since upon irradiation with the light of wavelength of 360 nm it is irreversible reaction, the first-order

equation was used for it description. Similar procedure yielded its kinetic parameters.

Acknowledgements

P.K. and M.M would like to thank The Wrocław Research Centre EIT+ for financial support within the frame of the project "Biotechnologies and advanced medical technologies" – BioMed (POIG.01.01.02-02-003/08) financed from the European Regional Development Fund (Operational Programme Innovative Economy, 1.1.2).

References

1. Christenson, S. D.; Liu, W.; Toney, M. D.; Shen B. *J. Am. Chem. Soc*. **2003**, *125*, 6062-6063, http://dx.doi:10.1021/ja034609m
2. B. Perman, B.; Srajer, V.; Ren. Z.; Teng, T.; Pradenvart, C.; Ursby, T.; Bourgeois, D.; Schotte, F.; Wulff, M.; Kort, R.; Hellingwerf, K.; Moffat, K. *Science* **1998**, *279*, 1946-1950, http://dx.doi:10.1126/science.279.5358.1946
3. Willey, J. M.; van der Donk, W. A. *Annu. Rev. Microbiol*. **2007**, *61*, 477-501. http://dx.doi:10.1146/annurev.micro.61.080706.093501
4. Babica, P.; Blaha, L.; Marsalek, B. *J. Phycol*. **2006**, *42*, 9-20. http://dx.doi:10.1111/j.1529-8817.2006.00176.x
5. Gulledgea, B. M.; Aggen, J. B.; Huang, H. B.; Nairn, A. C.; Chamberlin, A. R. *Curr. Med. Chem*. **2002**, *9,* 1991-2003. http://dx.doi:10.2174/0929867023368845
6. Edwards, J. V..; Lax, A. R.; Lillehoj, E. B.; Boudreaux, G. J. *J. Agr. Food Chem.* **1987**, *35*, 451-456, http://dx.doi:10.1021/jf00076a003
7. Tsuge, T.; Harimoto, Y.; Akimitsu, K.; Ohtani, K.; Kodama, M.; Akagi, Y.; Egusa, M.; Yamamoto, M.; Otani, H*. FEMS Microbiol. Rev.* **2013**, *37*, 44-66. http://dx.doi:10.1111/j.1574-6976.2012.00350.x
8. Kanoh, K .; Kohno, S.; Katada, J.; Hayashi, Y.; Muramatsu, M.; Uno, I. *Biosci. Biotech. Biochem.* **1999**, *63*, 1130-1133. http://dx.doi:10.1271/bbb.63.1130
9. Kim, M. Y.; Vankayalapati, H.; Shin-Ya, K.; Wierzba, K.; Hurley, L. H. *J. Am. Chem. Soc*. **2002**, *124*, 2098-2099. http://dx.doi:10.1021/ja017308q
10. Martinek, T. A.; Fülöp, F. *Chem. Rev.* **2012**, *41*, 687-702 http://dx.doi:10.1039/c1cs15097a
11. Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252-262. http://dx.doi:10.1038/nchembio876
12. Jewgiński, M.; Latajka, R.; Krężel, A.; Haremza, K.; Makowski, M.; Kafarski, P. *J. Mol. Struct.* **2013**, *1053,* 129-139. http://dx.doi:10.1016/j.molstruc.2012.08.042

13. Jewgiński, M.; Krzciuk-Gula, J.; Makowski, M.; Latajka, R.; Kafarski, P. *J. Org. Chem.* **2014**, *10*, 660-666. http://dx.doi:10.3762/bjoc.10.58
14. Siodłak, D.; Macedowska-Capiga, A.; Broda, M. A.; Kozioł, A. E.; Lis, T. *Peptide Sci.* **2012**, *98*, 466-478. http://dx.doi:10.1002/bip.22082
15. De Marco, R.; Greco, A.; Rupiani, S.; Tolomelli, A.; Tomasini, C.; Pieraccini, S.; Gentilucci, L. *Org. Biomol. Chem.* **2013**, *11*, 4316-4326. http://dx.doi:10.1039/c3ob40357b
16. Viola, A.; Terrazzano, L.; Cerisoli, L.; Caldi, J.; Tolomelli, A. *Eur. J. Org. Chem.* **2016**, *19*, 3217-3222. http://dx.doi:10.1002/ejoc.201600376
17. Ferreira, P. M.; Maia, H. L. S.; Monteiro, L. S.; Sacramento, J. *J. Chem. Soc., Perkin Trans. 1*, **1999**, 3697-3703. http://dx.doi:10.1039/A904730A
18. Latajka, R.; Jewgiński, M.; Makowski, M.; Pawełczak, M.; Huber, T.; Sewald, T.; Kafarski, P. *J. Pept. Sci.* **2008**, *14,* 1084-1095. http://dx.doi:10.1002/psc.1045
19. Kometani, M.; Ihara, K.; Kimura, R.; Kinoshita, H. *Bull. Chem. Soc. Jpn*. **2009**, *82*, 364-389. http://dx.doi:10.1246/bcsj.82.364
20. Latajka, R.; Makowski, M.; Jewgiński, M.; Pawełczak, M.; Koroniak, H.; Kafarski, P. *New J. Chem*. **2006**, *30*, 1009-1018. http://dx.doi:10.1039/B601634K
21. Dugave, C.; Demange, L. *Chem. Rev*. **2003**, *103*, 2475-2532. http://dx.doi:10.1021/cr0104375
22. Kubica, Z.; Koźlecki, T.; Rzeszotarska, B. *Chem. Pharm. Bull*. **2000**, *48*, 296-297. http://dx.doi:10.1248/cpb.48.296
23. Inai, Y.; Kurashima, S.; Hirabayashi, T.; Yakota, K. *Biopolymers* **2000**, *53*, 484-496. http://dx.doi:10.1002/(SICI)1097-0282(200005)53
24. Shigematsu, N.; Kayakiri, N.; Okada, S.; Tanaka H. *Chem. Pharm. Bull.* **1997**, *45*, 236-242. http://dx.doi:10.1248/cpb.45.236
25. Maekawa, K.; Igarashi, T.; Kubo, K.; Sakurai, T. *Tetrahedron* **2001**, *57*, 5515-5526. http://dx.doi:10.1016/S0040-4020(01)00477-X
26. H. Hoshina, H.; Kubo, K.; Morito, A.; Sakurai, T. *Tetrahedron*, **2000**, *56*, 2941-2951. http://dx.doi:10.1016/S0040-4020(00)00188-5

